AD NUMBER

AD293207

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational use; Dec 1962. Other requests shall be referred to Army Biological Labs, Frederick MD.

AUTHORITY

SMUFD D/A ltr 4 Feb 1972

FLUOROCHROME STUDIES OF MICROORGANISMS IN LIQUID MEDIA

TRANSLATION NO.
700

December 1962

U.S. ARMY BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

Chemical Corps Biological
Laboratories: FD2-3742, T-18-1
JPRS: R-2874

3 December 1962

FLUOROCHROME STUDIES OF MICROORGANISMS IN LIQUID MEDIA

U. S. JOINT PUBLICATIONS RESEARCH SERVICE
BUILDING T-30
OHIO DRIVE & INDEPENDENCE AVENUE, S.W.
WASHINGTON 25, D. C.

FLUOROCHROME STUDIES OF MICROORGANISMS IN LIQUID MEDIA [*]

[Summary: In a letter sent by the Battelle Institute, Frankfurt, to the CAP Company on 18 May 62 an interim report is given on fluorochrome studies of microorganisms in physiological saline solution. It was found that 16 fluorochromes produce fluorescent staining of microorganisms, with an especially high degree of differentiation achieved with Acridine Yellow and Acridine Orange. The ground fluorescence of the fluorochromes is so slight that it has no appreciable effect on the test results. Further tests are in progress on the effect of osmotic pressure, pH and fluorochrome concentration.]

Dear Sirs:

We wish to present herewith our second report dealing with the experimental results of the above-mentioned investigation carried out during the months of February through April 62. The preparation of this report has unfortunately been delayed due to various illnesses and we apologize for this delay.

As was anticipated in our work plan of 27 Oct 61 we began our studies in the period covered by the report with experiments in a liquid medium, after having carried out smear-fluorochromations in the first experimental period. The experiments of the second period are still in progress. Hence this interim report will contain no final results: these as well as our final conclusions will be included only in our next report.

The studies relating to fluorochromation in liquid medium began with the fluorochrome-film test series. The microorganisms were placed, in a physiological salt solution, on microscope slides previously treated with various fluorochromes. The method of preparation of these slides was as follows:

Starting from a one-percent alcoholic stock solution of the fluorochrome in question dilute solutions were prepared which were then placed on the thoroughly cleaned slide and then poured off after one minute. In this way the slide was coated with an extremely thin, almost invisible film of fluorochrome. Slides prepared in this manner may be preserved for a very long time.

[*] Text of letter, Reference 101-11/HE/den, 18 May 62, from Battelle Institute, Frankfurt, to CAP, Frankfurt (Main).

A drop of the bacterial suspension to be tested was placed on the slide, covered with a glass cover and the coloration of the bacteria was directly observed.

On the basis of experiences acquired during the first experimental period only those of the previously employed fluorochromes were used in the present experiments which had been found to be basically suitable for these experiments. The microorganisms investigated were the same as in the first experimental period: Staphylococcus albus, Sarcina lutea, Serratia marcescens, Bacillus subtilis, Clostridium botulinum and Aspergillus niger.

The results of this test series with living microorganisms in liquid medium may be summarized as follows: Of the 35 fluorochromes initially used 16 were basically suitable for fluorochromation under these conditions. They are:

Acridine Yellow	Entozone Granulate
Acridine Orange	Euchrysine
Auramine-O	Morin 1 (Chroma)
Aurophosphine-O	Morin 2 (Merck)
Brilliant Dianil Green	Rivanol
Coriphosphine-O	Thioflavine-S
Coriphosphine-Fuchsin	Thioflavine-T
Diamond Phosphine	Trypaflavine

All the above fluorochromes cause the microorganisms to fluoresce clearly, the color of fluorescence being either green, greenish yellow, yellow or orange. An especially high degree of differentiation is achieved with the acridine dyes. Whether the individual microorganisms are colored to a differentiable extent cannot as yet be stated with certainty.

In our final report to be issued at a later date we shall present the results in the form of clear color charts.

In the following test series we used the 16 fluorochromes which had been found to be particularly suitable for these experiments, that is, which exhibited a relatively good staining of the microorganisms in presence of a relatively slight ground fluorescence (fluorescence of the solution of the dyestuff proper). For the fluorochrome experiments the bacteria, suspended in physiological saline solution, were mixed with the fluorochrome in question (also dissolved in physiological saline solution) in a 1:1 ratio. The original concentration of the fluorochrome was 1:1,000, hence the final concentration was 1:2,000. Immediately after the addition of the fluorochrome a drop of the test mixture was transferred to the slide. The fluorochromes Morin 1, Morin 2 and Rivanol were dissolved in distilled water and not in physiological saline solution.

The same experimental arrangement was employed in another test

series, in which the final concentration was 1:4,000.

The results of both these experimental series may be summarized as follows:

The bacteria are clearly stained by all the fluorochromes investigated, but Aspergillus niger is represented only very poorly. In most cases there occurs a green, greenish yellow, yellow or orange fluorescence of the microorganism, in certain cases only a green or yellow fluorescence. Disregarding small differences the coloration at 1:4,000 end concentration is just as strong as at a concentration of 1:2,000.

Of particular significance was the question whether and to what extent the ground fluorescence of the given dyestuff influences the representation of the corpuscular element. In this connection we came to the conclusion that with the exception of Trypaflavine the ground fluorescence of the individual fluorochrome is so slight that it exerts no observable effect on the test results under the experimental conditions employed. This is especially evident in the case of lower concentrations.

As was already mentioned, the experiments have not yet been concluded. At the present time tests are in progress aiming at the clarification of the influence of the osmotic pressure, the pH value and the fluorochrome concentration. At the same time we are investigating whether a washing-out of the fluorochrome is possible or whether the extinction of the fluorescence can be brought about in any other way.

As a supplement to this report we are including herewith a color chart showing the results of the first experimental phase in a tabulated manner. The fluorescence colors after two and 20 minutes of staining (smear preparation) are reproduced for each microorganism. The number of crosses is a measure of the intensity of the fluorescent light.

We shall present all subsequent results, if possible, in this manner and included in future interim reports; in our final report all results will be summarized and discussed. This last report will include also the fluorescence-microscopic photographs which will have been taken in the interim of the most interesting preparations.

We expect that the current studies will produce further important data leading to the solution of the problem at hand.

Sincerely yours,

BATTELLE-INSTITUT E.V.

Dr. H. Eder

Enclosure.

P.S.: We are sending a copy of this report to Dr Mutschin of the Department of Defense (Bundesministerium der Verteidigung), Bonn, and to Dr Rodewald of the Federal Office of Military Engineering and Procurement (Bundesamt fur Wehrtechnik und Beschaffung), Koblenz (Conference of 5 Jan 61).

[Key to Color Chart:

1) Fluorochrome, 1:1,000 aqueous solution
2) Acridine Yellow
3) Brilliant Dianil Green
4) Chromotropic Acid
5) Cresyl True Violet
6) Cresyl True Violet V
7) Fuchsin for Bacillus Staining
8) Basic Fuchsin
9) Fuchsin S (Acid Fuchsin)
10) Fuchsin S-Methylene Blue
11) Magdala Red, True
12) Malachite Green
13) Methylene Blue
14) Methyl Green
15) Methyl Green OO
16) Neutral Red B Extra
17) Rosol Red B
18) Thiazine Red R
19) Thiazole Yellow]